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13. ABSTRACT ***(Maximum 200 Words)***

Hybrid transgenic/genetically obese mice have been bred to evaluate the effects of obesity and weight gain on mammary tumor development. In the TGF-α/*Lep*^{ob} Incidence Study, *Lep*$^{+}$*Lep*$^{+}$ (homozygous) lean, *Lep*$^{+}$*Lep*^{ob} (heterozygous) lean, and *Lep*^{ob}*Lep*^{ob} (homozygous) obese mice are being followed. The obese mice weigh more than the lean mice. Also, the heterozygous weigh more than the homozygous lean mice, and have a higher incidence of mammary tumors. The obese mice are not old enough to evaluate tumor development. In an additional study, the effect of weight-cycling on mammary tumor development is being evaluated using TGF-α/*Lep*$^{+}$*Lep*^{ob} mice. Results will be compared to *ad libitum* fed and pair-fed control groups. These mice are just reaching the age of tumor detection. We are also assessing the results of diet-induced obesity using TGF-α/*Lep*$^{+}$*Lep*$^{+}$ mice. The mice are falling into obese and diet resistant groups. Tumors have been found in the two heaviest obese mice. A second hybrid strain, TGF-α/*Lepr*^{db} has also been bred. The *Lepr*^{db} Incidence Study has completed enrollment of the two lean groups. As described above for the *Lep*^{ob} strain, the TGF-α/*Lepr*$^{+}$*Lepr*^{db} heterozygous weigh more than the homozygous *Lepr*$^{+}$*Lepr*$^{+}$ lean mice. These mice are not old enough for tumors yet.

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FOREWORD

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TABLE OF CONTENTS

INTRODUCTION:

Breast cancer is the most frequently diagnosed cancer in women (1). One risk factor that has been proposed to play a role in the development of postmenopausal breast cancer is increased body weight and/or body mass index (BMI) (weight/height^2). Several reviews of the literature have shown that both case-control and prospective studies support this conclusion (2,3). A recent study of Italian women concluded that overweight accounted for 10.2% of postmenopausal breast cancer cases (4). Other studies have implicated weight gain as a risk factor for breast cancer development (5-8). However, body weight and weight gain in breast cancer development have not been supported by all studies. This may be partially attributable to technical factors. For example, conflicting results from human studies might be due to inaccurate recalled body weight. Also, whether weights from before or after menopause are used makes a difference in the conclusions drawn (9). There are other confounding factors such as the influences of ethnic and social backgrounds, and the potential for interactions with other risk factors.

Animal models are frequently used to study human diseases. Currently, the number of published studies investigating the role of obesity and mammary tumorigenesis in animals is limited. However, these studies (10-17) indicate that obese mice and rats have been found to have a higher incidence of either chemically-induced or spontaneous mammary tumors. Two additional publications using weight-cycled rats further implicate weight gain as an important factor in the development of chemically-induced mammary tumors (18,19). In dogs lower body weight appears to protect against mammary tumor development (20) while obesity in dogs has been reported to increase the risk of mammary tumors (21). Clearly, the role of these factors as potential risk factors in the etiology of human breast cancer is an important issue to resolve.

We have proposed that the role of body weight, BMI and weight gain in the development of breast cancer can be addressed systematically in a physiologically relevant animal model, *i.e.*, transgenic mice. Our hypothesis is that weight gain and the accompanying metabolic changes create a milieu conducive to enhanced development of oncogene-induced mammary tumors. We are developing a molecularly well-defined animal model to test this hypothesis. "Hybrid" obese-transgenic mice are being produced by mating strains of genetically obese mice in which the molecular defect has been identified, *i.e.*, *Lep^{ob}* or *Lepr^{db}*, with a transgenic mouse line overexpressing TGF-α. This proto-oncogene has been implicated in the etiology of human breast cancer (22,23), and its presence in mice has been demonstrated to result in mammary tumors (24). These hybrid mice are being used to systematically evaluate the role of body weight and weight gain in the development of mammary tumors in genetically obese, dietary obese, and lean mice.

BODY:

Our goals for year two of this project as outlined in the Statement of Work (Appendix A) were to 1) complete enrollment of TGF-α/*Lep^{ob}* strain mice into the incidence study and monitor body weight and tumor development; 2) collect samples from euthanized mice from the incidence study; 3) enroll TGF-α/*Lep^{ob}* strain mice in the weight-cycling study; 4) follow weekly food intake and body weight measurements of mice in the weight-cycling study and monitor tumor development; 5) enroll TGF-α/*Lep^{ob}* strain mice in diet-induced obesity study; 6) order *Lepr^{db}*

strain mice, initiate breeding colony and cross to TGF-α mouse strain; 7) enroll TGF-α/*Lepr*^{db} strain mice in incidence study. In general, these goals have been met with some modifications that will be described below.

1-Complete enrollment of TGF-α/*Lep*^{ob} strain mice into the incidence study and monitor body weight and tumor development and 2-Collect samples from euthanized mice from the incidence study.

A total of 39 homozygous *Lep*$^{+}$*Lep*$^{+}$ lean mice, 41 heterozygous *Lep*$^{+}$*Lep*^{ob} lean mice, and 60 homozygous *Lep*^{ob}*Lep*^{ob} obese mice were enrolled in the incidence study (all mice contained the TGF-α gene). As indicated in last year's report we included the heterozygous mice in this regimen when we found out that the mice carrying TGF-α would not lactate, and thus we could not use these mice for breeding purposes as originally planned. As indicated above, more obese then lean mice were enrolled in the study. This was due to the excess mortality rate of the obese mice we first enrolled in the study. When obese mice were housed individually they had great difficulty grooming themselves as they aged, and many of them developed open skin lesions and had to be sacrificed. We have subsequently housed all the mice in this study in pairs. The obese mice have done much better although they still appear to be dying at an accelerated rate compared to the two lean groups.

The growth curves for the three genotypes of the TGF-α/*Lep*^{ob} mouse strain are included in Figure 1 (Appendix B). Body weights are presented by 4-wk intervals. Body weights are statistically different at all time points among the three groups when analyzed by ANOVA. Post hoc analysis indicated that, as expected, the obese mice were heavier than the two lean groups. The two lean groups were not statistically different from each other by this analysis, but, when analyzed by a Student's "t" test all comparisons between the two lean groups were different with heterozygous mice weighing more than homozygous lean mice.

Mammary tumors have developed in 9 of 41 heterozygous TGF-α/*Lep*$^{+}$*Lep*^{ob} lean mice. Tumors were initially found between 48 and 78 wk of age. No mammary tumors have been found in either the homozygous lean or homozygous obese groups. Summaries of numbers of live and dead mice are presented in Table 1 (Appendix C). Blood samples have been obtained from most of the mice that were euthanized and tissue samples have been delivered to Dr. Joseph Grande, co-investigator, a pathologist at Mayo Clinic. We have also obtained serum and tissue samples from nontransgenic mice of the three genotypes that were 13-14 months of age when euthanized to provide "control reference" data. Initial analysis of mammary tumors indicates that they are primarily moderately differentiated adenocarcinomas.

3-Enroll TGF-α/*Lep*$^{+}$*Lep*^{ob} mice in weight-cycling study and 4-Follow food intake, body weights and tumor development.

TGF-α *Lep*$^{+}$*Lep*^{ob} mice have been enrolled in the weight-cycling study. Due to the problems we have had with housing the obese mice individually and the uncertainty at this time as to when and if they will develop mammary tumors we have not yet included them in this protocol as yet. Three groups of lean mice are being used as described in the original protocol. The *ad libitum* fed group (n=30) is fed purified diet C Table 2 (Appendix D) on an *ad libitum* basis. This diet is

based on AIN93 recommendations for maintenance feeding of mice and rats (25). The weight-cycling group (n=30) is fed purified diet D Table 2 (Appendix D) for 3 wk at 50% of the intake of the *ad libitum* fed group. This diet is isocaloric with Diet C but has twice the protein, vitamin, mineral and fat content in order that the two groups of mice receive an equivalent amount of these nutrients. The major difference is calories due to carbohydrate intake. Following the 3 wk food restriction period, the weight-cycled mice are then fed Diet C *ad libitum* for 3 wk. The oldest mice in the study are now in their 8^{th} cycle. An additional control group (n=33) is chronically food restricted a mixture of DIET C and D (2:1) to match the intake of the weight-cycled mice for each 6 wk interval. By necessity this group was enrolled to follow the weight-cycled group.

Body weights for the mice in this study are shown in Figure 2 (Appendix E). As can be seen the general pattern is that the weight-cycled mice lose weight during the first week of food restriction, then their body weights plateau. When *ad libitum* feeding is instituted the mice rapidly regain the lost weight. Due to the small number of mice in the food restricted group statistics have been done only to week 27. For the first two weeks of the study (9 and 10 wk of age) there were no differences in body weights among the three groups. Following the initiation of weight cycling and food restriction, there were significant differences in body weights among the three groups (11-13 wk of age). With refeeding weight was rapidly regained, and at 14 wk of age there were no differences among the three groups. This pattern was more or less followed for the two additional cycles were statistics have been calculated. At weeks 26 and 27 there were no differences in body weights among the three groups.

To date two mice in the *ad libitum* fed group have developed tumors at 48 and 52 wk of age and were both euthanized at approximately 55 wk of age. One additional *ad libitum* mouse was euthanized due to an injury received when her leg got stuck in the cage.

5-Enroll mice in diet-induced obesity study.

We have enrolled 25 mice in the chow-fed group and 51 in the group fed the condensed milk-corn oil-chow (CCC) diet. All mice were homozygous (TGF-α/*Lep*$^{+}$*Lep*$^{+}$) lean females. It has previously been reported that rats fed this diet either overeat and become obese or restrict their intake and remain at a body weight similar to that of chow fed rats (26). This resulted in the definition of diet-induced obesity and diet-resistant rats. This diet has also been used in short-term studies to identify strains of mice susceptible toward dietary induced obesity (27). Mice were started on the CCC diet at 10-11 wk of age and presently range from 14 to 50 wk of age.

Body weight data for mice that have been on the diet for 14 wk are shown in Figure 3 (Appendix F). At this point there were 21 chow fed mice and 39 mice fed the CCC diet. The CCC mice were divided into three groups each with 13 mice based on body weight ranking. The heaviest group was designated DIO (diet-induced obese), the group in the middle was designated MID (middle range) and the lightest mice were designated DR (diet resistant). As can be seen in Figure 3, statistical analysis indicated that the DIO mice weighed the most followed by the CHOW and MID mice that weighed a similar amount, and the DR group weighed significantly less than the three other groups.

Tumors have developed to date in two DIO mice. One of these mice was 49 wk of age when the tumor was detected. The mouse weighed 44.5 g and was the second heaviest of the 11 mice that have reached that age. The second mouse's tumor was detected at 41 wk of age when the mouse weighed 49.3 g. This was the heaviest of the 22 mice that have reached this age point. We anticipate that this protocol should provide very interesting results since the mice fed the CCC diet will have different body weights and weight gain but do not have different types of diet while the chow and MID mice have similar body weights but different diets. These results should help to clarify if high fat diets and/or body weight/weight gain are significant factors in mammary tumor development.

6- Order *Lepr^{db}* mice, initiate breeding colony and cross to TGF-α mouse strain.

A recent paper (28) indicated that the *db* mouse strain that we had planned to buy from Jackson Laboratory could potentially have growth problems related to the gene for misty. After having spoken with Dr. Gary Truett and evaluating the information on hand, we have decided to use *Lepr^{db}/Lepr^{db}* without the misty gene that Dr. Truett and his coworkers have developed. These mice arrived in August of 1998. Dr. Truett also provided an assay to genotype the mice and this has been set up in the laboratory.

The *Lepr^{db}* mouse strain was successfully cross-bred with the TGF-α mouse strain using a similar strategy as that previously described for the TGF-α/*Lep^{ob}* mice, *i.e.*, nontransgenic female lean (either *Lepr^{+}Lepr^{+}* or *Lepr^{+}Lepr^{db}*) mice are mated with TGF-α male mice that are either *Lepr^{+}Lepr^{+}* or *Lepr^{+}Lepr^{db}*.

We have completed enrollment of the TGF-α/*Lepr^{+}Lepr^{+}* homozygous lean group (n=40) and have 39 mice enrolled in the TGF-α/*Lepr^{+}Lepr^{db}* heterozygous lean group. In the TGF-α/*Lepr^{db}Lepr^{db}* obese group 11 mice have been enrolled but one has already died. Body weight curves for the three groups are shown in Figure 4 (Appendix G). At the four-week intervals analyzed with all three groups, 6, 10 and 14 wk of age, there was a significant difference by ANOVA. At both 6 and 10 wk of age all three groups were also significantly different from each other by Newman-Keul post hoc analysis. When the two lean groups were analyzed by Student's "t" test significant differences were found at 6, 10, and 14 wk of age but not at the older ages where the n values for each group were substantially smaller.

Since the homozygous lean group is filled we have started enrolling mice in a DIO study with this genotype, and as soon as the heterozygous group is filled we will enroll those mice in the weight-cycling regimen. We are also saving some nontransgenic mice from the three genotypes to serve as controls.

KEY RESEARCH ACCOMPLISHMENTS:

- Established TGF-α /*Lep*^{ob} mouse strain.
- Determined that low level leptin treatment restores fertility to young male obese mice.
- Have preliminary observations that TGF-α/*Lep*$^{+}$*Lep*^{ob} heterozygous lean mice weigh more than the lean homozygous mice and have a much higher incidence of mammary tumors.
- Mice without the obese, *Lep*^{ob} gene, *i.e.*, homozygous *Lep*$^{+}$*Lep*$^{+}$ lean mice are susceptible to diet-induced obesity when they consume a diet of condensed milk-chow-corn oil.
- Established TGF-α/*Lepr*^{db} mouse strain.
- Have established a collaboration with Drs. Aminah Jatoi, an oncologist at Mayo Clinic, and Dr. Phuong Nguyen, a pathologist at the University of Minnesota, to evaluate aspect of muscle protein metabolism in tumor bearing mice.

REPORTABLE OUTCOMES

Invited speaker, "An Animal Model to Study the Effect of Obesity on the Development of Mammary Tumors," Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN October 1998

Invited speaker, "Development of an Animal Model to Investigate the Relationship Between Body Weight and Breast Cancer," Department of Food Science and Nutrition, University of Minnesota, St. Paul, MN December 1998.

Invited speaker, "Body Weight and the Development of Breast Cancer," Austin Rotary Club, Austin, MN March 1999.

Presented a poster, "Development of a New Mouse Model to Study the Effect of Obesity on the Development of Mammary Tumors," Toxicology Society Symposium – Role of Diet and Caloric Intake in Aging, Obesity, and Cancer, Reston, VA October 1998. (Appendix H)

Presented a poster, "Restoration of Fertility in Young Adult Male TGF-α *Lep*^{ob}*Lep*^{ob} Hybrid Mice with Lose Dose Leptin Treatment," Experimental Biology, Washington, D.C. April 1999 (FASEB J., 13 A927, 1999). (Appendix I)

Restoration of Fertility in Young Obese (*Lep*^{ob}*Lep*^{ob}) Male Mice With Low Dose Recombinant Mouse Leptin Treatment.
Margot P. Cleary, Heidi L. Bergstrom, Tina L. Dodge, Susan C. Getzin, Michelle K. Jacobson, and Frederick C. Phillips.
To be submitted – 10/99

CONCLUSIONS:

This research project is going along very well. We have met our goals for the first two years of the project and are now starting to see some exciting results. The most interesting finding to date is that there appears to be a heterozygous effect of the presence of both the *Lep*^{ob} and *Lepr*^{db} gene in lean mice. Furthermore, in the TGF-α/*Lep*$^{+}$*Lep*^{ob} mice we are now seeing that this increase in body weight is associated with an increased risk of mammary tumor development compared to the TGF-α/*Lep*$^{+}$*Lep*$^{+}$ lean mice. It remains to be determined if body fat in these mice is also higher. Further analysis of tissues and blood samples will hopefully shed further insight into this finding. The observation that some homozygous lean mice can become obese when challenged with a high-fat diet is also of interest. This is the first report that mice respond to this diet in a similar manner as do several strains of rats, and the finding is even further intriguing because these mice are homozygous lean. We are also planning to evaluate the effects of this dietary intervention in mice that are heterozygous lean. This model in the end may have much greater applicability to humans than the genetically obese mice will.

REFERENCES:

1. C.C. Boring, T.S. Squires, T. Tong, S. Montgomery, *CA Cancer J. Clin.* **44**, 7 (1994).

2. R. Ballard-Barbash, *Cancer* **74**, 1090 (1994).

3. M.P. Cleary, N.J. Maihle, *Proc. Soc. Exp. Biol. Med.* **216**, 28 (1997).

4. M. Mezzetti, *et al, J. Natl. Cancer Inst.* **90**, 389 (1998).

5. D. Barnes-Josiah, J.D. Potter, T.A. Sellers, J.H. Himes, *Cancer Causes Control* **6**, 112 (1995).

6. E. Taioli, J. Barone, E.L. Wynder, *Eur. J. Cancer* **31A**, 723 (1995).

7. N.B. Kumar, G.H. Lyman, K. Allen, C.E. Cox, D.V. Schapira, *Cancer* **76**, 243 (1995).

8. C. Magnusson, *et al, Int. J. Cancer* **76**, 29 (1998).

9. L.C. Yong, C.C. Brown, A. Schatzkin, C. Schairer, *Am. J. Epidemiol.* **143**, 985 (1996).

10. G.L. Wolff, R.L. Kodell, A.M. Cameron, *J. Tox. Environ. Health* **10**, 131 (1982).

11. S.H. Waxler, P. Tabar, L.P. Melcher, *Cancer Res.* **13**, 276 (1953).

12. S.H. Waxler, *Am. J. Clin. Nutr.* **8**, 760 (1960).

13. S.H. Waxler, G. Brecher, S.L. Beal, *Proc. Soc. Exp. Biol. Med.* **162**, 365 (1979).

14. W.E. Heston, G. Vlahakis, *J. Natl. Cancer Inst.* **26**, 969 (1961).

15. W.E. Heston, G. Vlahakis, *J. Natl. Cancer Inst.* **40**, 1161 (1968).

16. G.L. Wolff, D. Medina, R.L. Umholtz, *J. Natl.Cancer Inst.* **63**, 781 (1979).

17. D.M. Klurfeld, *et al, Proc. Soc. Exp. Biol. Med.* **196**, 381 (1991).

18. S.R. Harris, A.E. Brix, J.R. Broderson, O.R. Bunce, *Proc. Soc. Exp. Biol. Med.* **209**, 231 (1995).

19. A.R. Tagliaferro, *et al, J. Nutr.* **126**, 1398 (1996).

20. E.G. Sonnenschein, L.T. Glickman, L.T. Goldschmidt, L.J. McKee, *Am. J. Epidemiol.* **133**, 694 (1991).

21. D.P. Alenza, G.R. Rutterman, L. Peña, A.C. Beynen, P. Cuesta, *J. Vet. Intern. Med* **12**, 132 (1998).

22. S.E. Bates, *et al, Mol. Endocrinol.* **2**, 543 (1988).

23. R.A. Walker, J.M. Varley, *Cancer Surv.* **16**, 31 (1993).

24. Y. Matsui, S.A. Halter, J.T. Holt, B.L.M. Hogan, R.J. Coffey, *Cell* **61**, 1147 (1990).

25. P.G. Reeves, F.H. Nielsen, G.C. Fahey, Jr., *J. Nutr.* **123**, 1939 (1993).

26. B.E. Levin, A.C. Sullivan, *Am. J. Physiol.* **253**, R475 (1988).

27. D.B. West, J. Waguespack, B. York, J. Goudey-Lefevre, R.A. Price, *Mamm. Genome* **5**, 546 (1994).

28. G.E. Truett, R.J. Tempelman, J.A. Walker, J.K. Wilson, *Am. J. Physiol.* **275**, R29 (1998).

APPENDIX A- ORIGINAL STATEMENT OF WORK

III.B.2.e. Statement of Work

Months 1-2. Order breeding animals, racks and initial supplies. Set up assays for trans-oncogene and *ob* determinations.

Months 3-5. Initiate first set of breedings and determine genotype of offspring.

Months 5-6. Initiate matings of double heterozygous mice. Enroll female homozygous lean (+/+) and obese (*ob/ob*) mice in breast cancer incidence study. Test for genotypes and take blood samples. Weigh experimental mice.

Months 6-12. Continue double heterozygous matings and start homozygous (**TT**) trans-oncogene matings. Continue to enroll female homozygous lean (+/+) and obese (*ob/ob*) mice from both mating groups in the incidence study. Test for genotypes and take blood samples. Weigh mice and examine for tumors weekly. Monitor tumor growth.

Months 13-18. Continue to monitor mice enrolled in the incidence study. Collect samples from ethanized mice. When 25 mice enrolled per lean and obese groups, inititate weight-cycling study.
Monitor those mice, take blood samples and prepare and feed special diets.

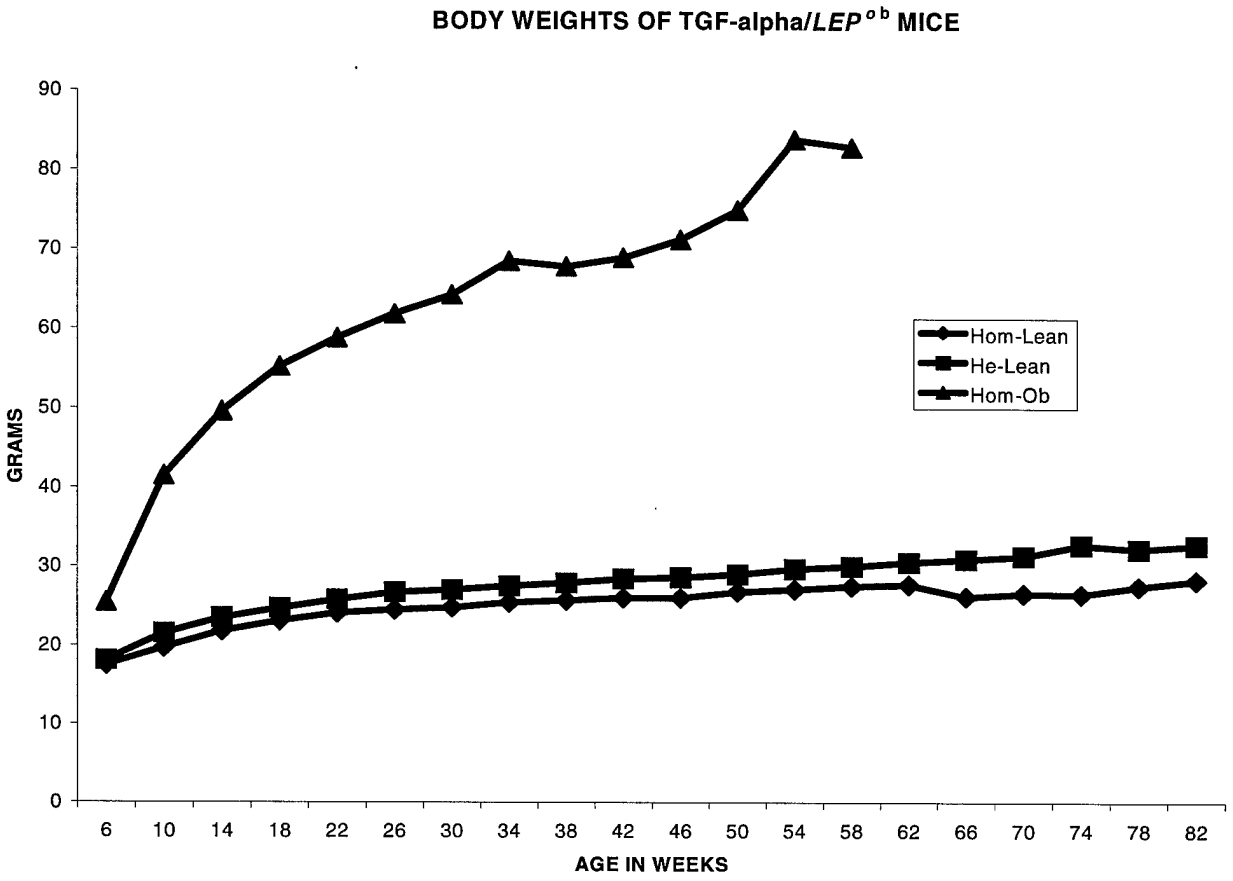
Months 15-18. Order *db* mice and inititate that breeding colony. Enroll *ob* mice in weight-cycling study and record weekly weights and food intakes and monitor tumor incidenc and growth in these mice. Prepare and feed special diets.

Months 18-21. Initiate matings of double heterozygous trans-oncogene *db* mice. Enroll female homozygous lean (+/+) and obese (*db/db*) mice in breast cancer incidence study. Test for genotypes and take blood samples. Weigh and monitor experimental mice and continue monitoring food intakes of weight-cycled mice. Continue diet preparations. Kill mice from the incidence study as they reach 16 months of age. Do body compositions on mice from incidence study. Enroll *ob* strain mice in diet-induced obesity study and monitor.

Montha 22-28. Continue heterozygous matings and start homozygous (**TT**) trans-oncogene matings of *db* mice colony. Continue to enroll *db* strain in incidence study and weigh mice, examine for tumors and monitor tumor growth of all experimenatal mice. Kill weight-cycled mice as they reach 16 months of age. Do cellularity and body compostion determinations. Kill mice from db incidence study and enroll additional animals in weight-cycling protocol.

Months 24-36. Record food intakes, body weight and monitor for tumors and tumor growth. Kill remaing weight-cycled mice and kill diet-induced obese *ob* mice as they reach 16 months of age. Perform cellularity measurements and body compostition analysis. Kill *db* mice from incidence study.

APPENDIX B- FIGURE 1



Body weight curves for TGF-α/*Lep*^{ob} mouse strain. Numbers of mice per group varies from 7 to 60 dependent upon age and genotype. Data were analyzed by ANOVA followed by Newman-Keuls post hoc analysis. At all time points ANOVA significantly different among the groups. Body weights of obese mice greater than body weights of the two lean groups by post hoc analysis at all time points. However, body weights of homozygous lean mice are significantly lower than heterozygous lean at all time points by Student's "t" test.

APPENDIX C -TABLE 1

Summary of TGF-α *Lep*^{ob} Mice (as of 8/31/99)

Group	# Enrolled	Present Age Range of Live Mice (wks)	Age When Mammary Tumor Detected (wks)	# With Mammary Tumors	# Dead	# Alive
Lep^{+}*Lep*$^{+}$	39	51-91		0	2[a]	37
Lep^{+}*Lep*^{ob}	41	62-92	48-78	9[b]	9	32
Lep^{ob}*Lep*^{ob}	60	23-61		0	21	39

[a]One of these mice had a malignant ovarian tumor.

[b]Initial pathological analyses of tumors indicated that these are adenocarcinomas.

APPENDIX D- TABLE 2

Composition of Diets to be Used in Weight Cycling Experiment (g/1000 g)

	Diet C[1*]	Diet D[2*]
Casein	140.0	280.0
L-Cystine	1.8	3.6
Corn Starch	470.692	322.8
Maltodextrin	160.0	98.0
Sucrose	100.0	61.0
Soybean Oil	40.0	80.0
Cellulose	40.0	59.584
Mineral Mix, AIN-93M-MX (TD 94049)	35.0	70.0
Vitamin Mix, AIN-93-VX (TD 94047)	10.0	20.0
Choline Bitartrate[3]	2.5	5.0
TBHQ (antioxidant)	0.008	0.016

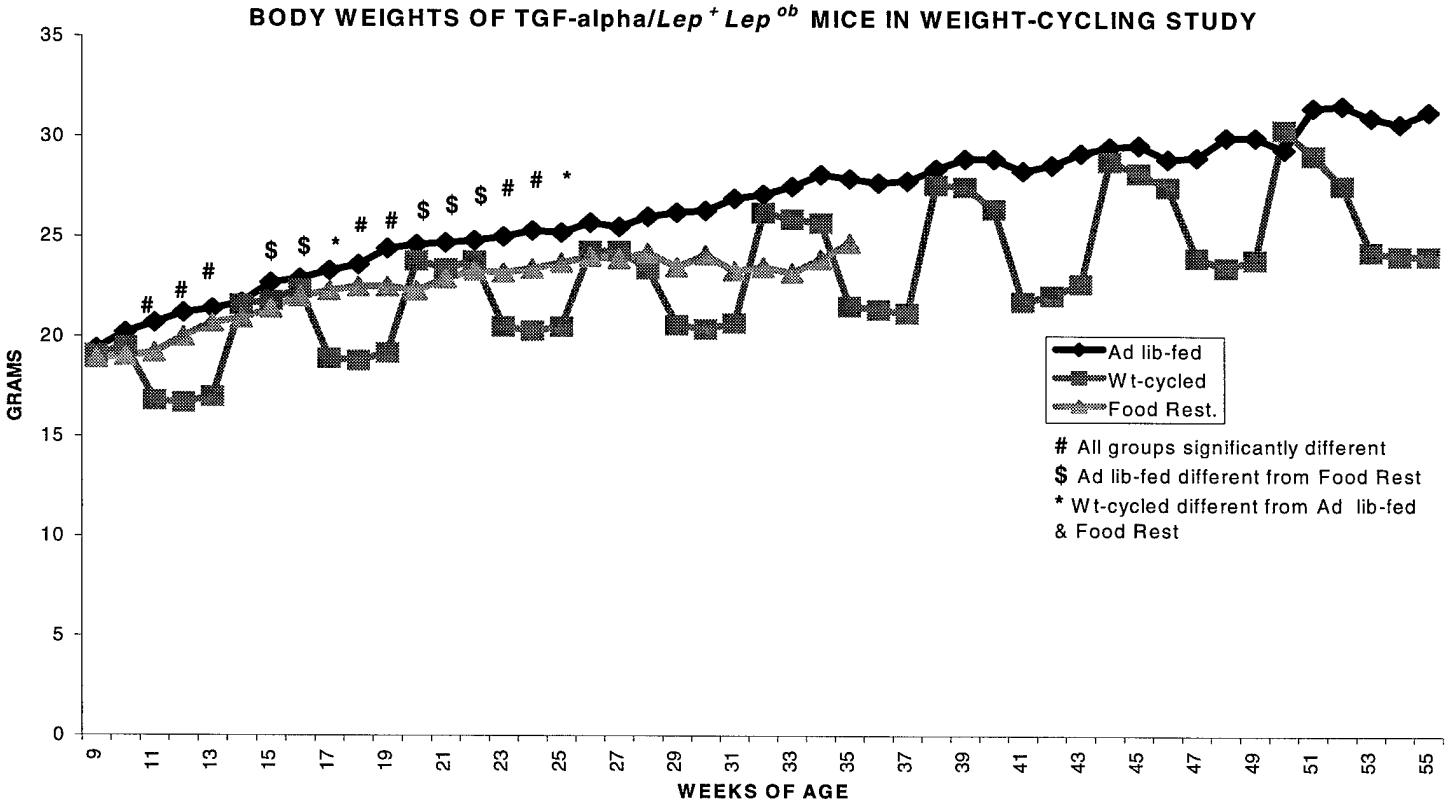
[1]This diet is a modification of the AIN-93 purified diet recommended for use in long-term rodent studies (25). Adjustments were made in carbohydrate and fiber sources to allow for it to be isocaloric with Diet D.

[2]This diet is designed to be isocaloric with Diet C and provide a similar amount of all nutrients except for carbohydrate when consumed at one-half of Diet C.

[*]Diets designed in consultation with Dr. Ronald Rose of Harlan Teklad.

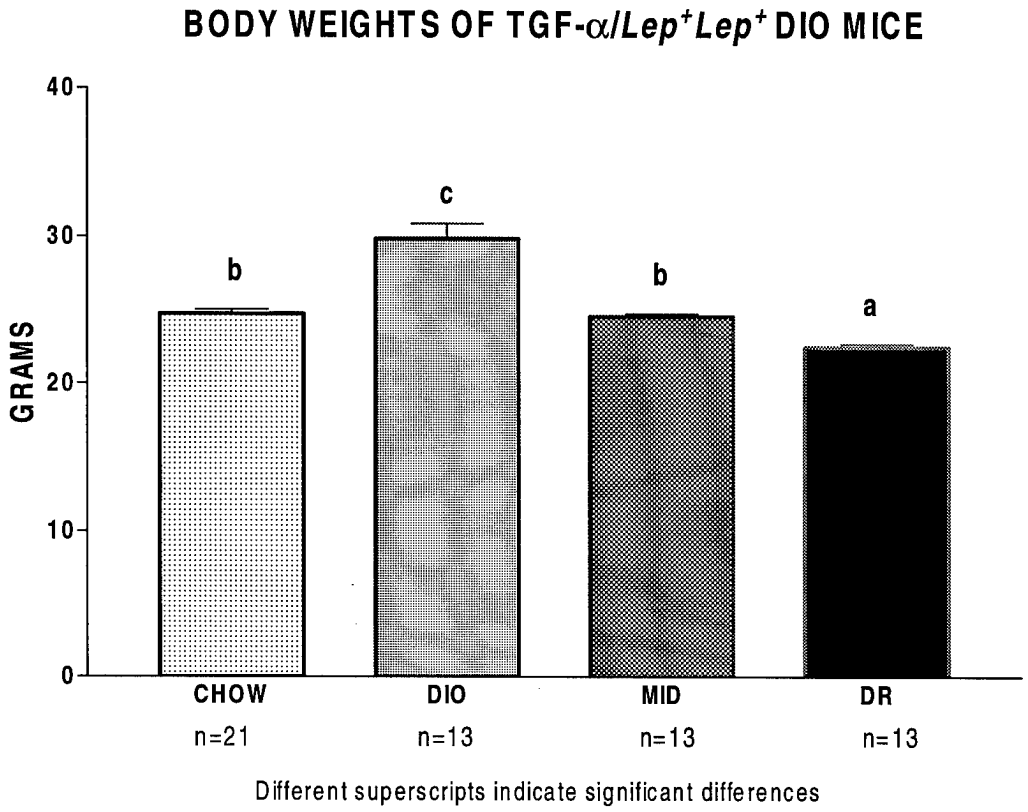
[3]41% choline.

APPENDIX E- FIGURE 2



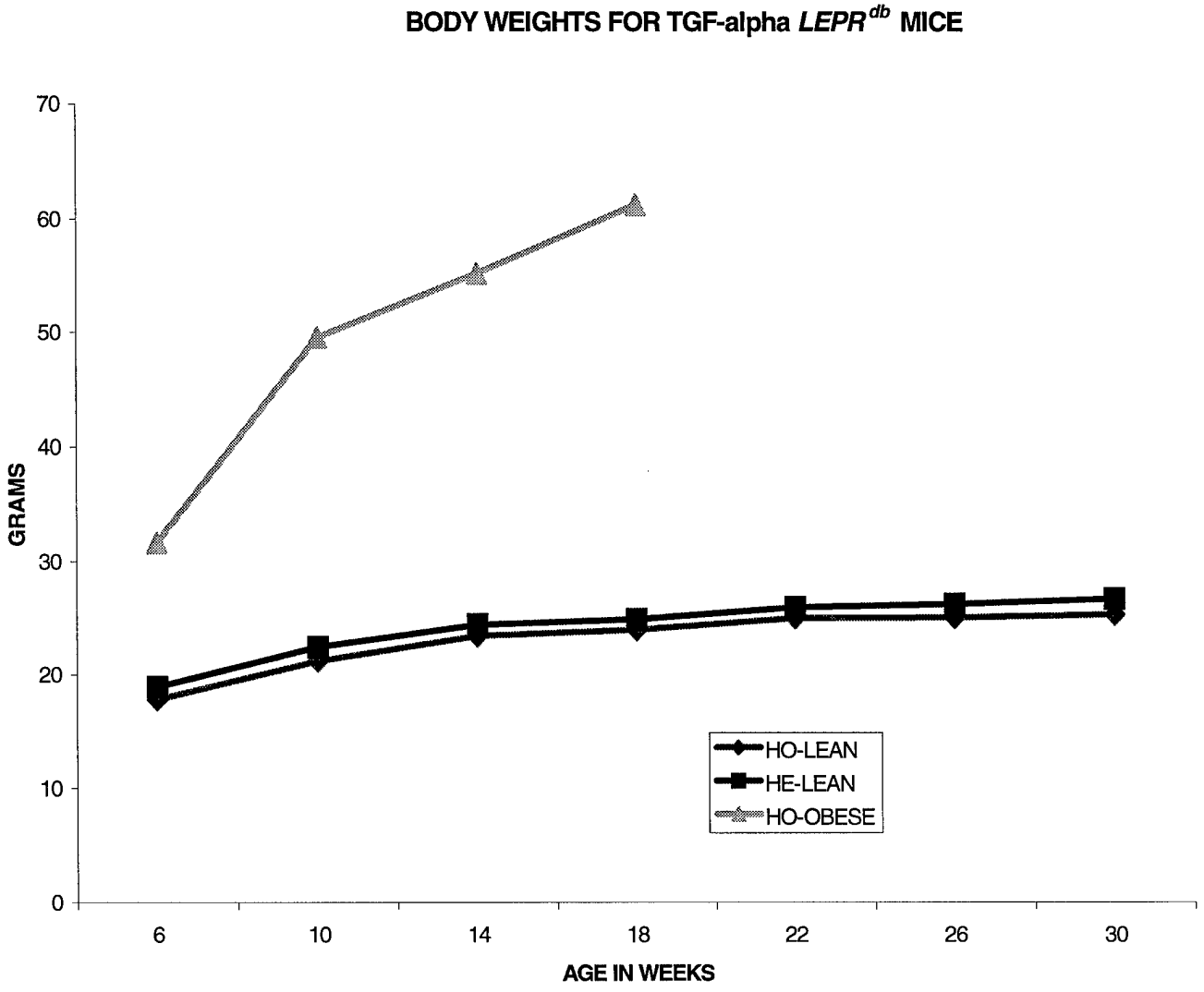
Body weights for TGF-α/*Lep*$^{+}$*Lep*^{ob} mice in weight-cycling experiment. Weeks of age 9-27 were analyzed by ANOVA followed by Newman-Keuls post hoc analysis. Number of mice per group varies from 18 to 33 dependent upon age and group. No symbol in this age range indicates no significant differences. # Indicates all groups are significantly different from each other, $ indicates ad libitum fed group different from food restricted group, * indicates weight-cycled group different from both ad libitum fed and food restricted group. Weeks 28 and higher were not statistically analyzed due to small numbers of mice in some groups.

APPENDIX F-FIGURE 3



Body weights of mice in DIO study at 24 weeks of age after 14 weeks on condensed milk-corn oil-chow diet. DIO mice are the heaviest 13 mice, MID are the 13 mice with body weights in the middle range, and DR are the 13 lightest mice. Data were analyzed by ANOVA followed by Newman-Keuls post hoc test. Chow group (n=21) fed Purina Rodent Chow 5001 throughout the study. Bars with different superscripts are significantly different from each other.

APPENDIX G-FIGURE 4



Body weights for TGF-α/*Lepr*^{db} strain mice. Data at weeks 6, 10 and 14 were analyzed by ANOVA followed by Newman-Keuls post hoc test. Obese mice weighed more than lean mice at these three time points. At 6 and 10 weeks the heterozygous *Lepr*$^{+}$*Lepr*^{db} mice weighed more than the homozygous *Lepr*$^{+}$*Lepr*$^{+}$ mice. At these three time points number of mice per group varied from 7 to 40. Body weights not analyzed after week 14.

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A NEW MOUSE MODEL TO STUDY THE EFFECT OF OBESITY ON THE DEVELOPMENT OF MAMMARY TUMORS. M P Cleary and N J Maihle. The Hormel Institute, University of Minnesota, Austin, MN and Tumor Biology Program, Mayo Clinic, Rochester, MN

Breast cancer is the most frequently diagnosed malignancy in women. Numerous studies have reported that the relative risk for postmenopausal breast cancer increases as body weight and/or body mass index (BMI) (weight/height^2) increases. Elevations in serum estrogens and/or insulin are characteristics associated with elevated body fat in obese women which may function as growth factors during breast tumor development. These and other factors that affect individuals over their life span are difficult to assess prospectively in humans. Although animal models are frequently used to investigate disease processes, there are few animal studies addressing the relationship between obesity and breast tumor formation. However, all these studies published to date indicate that both genetic and chemically-induced obesities decrease the latency and/or increase the incidence and tumor burden in rodents with either spontaneous or chemically-induced tumors. Recently we have developed a molecularly well-characterized model, *i.e.*, hybrid transgenic-obese mice that will be useful to study the interaction between body weight and the development of breast cancer. Transgenic mice overexpressing the oncogene, TGF-α, were crossbred with the genetically obese mouse strain, *Lep^{ob}*, in which the molecular defect responsible for obesity has been identified. Mating strategies include breeding nontransgenic female lean mice that are either homozygous (*Lep^{+}/Lep^{+}*) or heterozygous (*Lep^{+}/Lep^{ob}*) for leanness, because TGF-α female mice although fertile do not successfully lactate. The female mice are mated with TGF-α lean male mice of either genotype or with leptin treated homozygous (*Lep^{ob}/Lep^{ob}*) obese male mice. Offspring are genotyped for the transgene and with respect to *Lep* status. Groups of homozygous lean and obese and heterozygous lean female mice are currently being followed to determine how body fat, body weight, and weight gain interact to affect latency, incidence, and burden of mammary tumors.

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APPENDIX I

RESTORATION OF FERTILITY IN YOUNG ADULT MALE TGF-α *LEP^{ob}LEP^{ob}* HYBRID MICE WITH LOW DOSE LEPTIN TREATMENT.

M.P. Cleary, M.K. Jacobson, H.M. Bergstrom, F.C. Phillips, T.L. Dodge and S.C. Getzin. Hormel Institute, Univ of Minnesota, Austin, MN 55912

Previously, Mounzih et al (Endocrinology 138:1190-1193,1997) reported that treatment of adult ~70 g *Lep^{ob}Lep^{ob}* mice with 20µg/g bw human leptin restored fertility. We investigated whether a lower leptin dose could affect fertility of young adult 8-10 wk old ~43 g TGF-α *Lep^{ob}Lep^{ob}* hybrid male mice. In Experiment 1 (n=3) TGF-α *Lep^{ob}Lep^{ob}* male mice were treated daily with 5µg/g bw recombinant mouse leptin by ip injections for 10-16 days, and then with 2.5µg/g bw for 7-18 days. After the 1st wk mice were housed with 1-2 females for 5-7 day intervals. Females were observed to determine if pregnancy occurred. The mice lost weight and impregnated females (# pregnancies/# females, 3/6,5/6,5/10). In Experiment 2 2.5 µg/g bw leptin (n=4) was used for 34-38 days, and a vehicle-injected group (n=4) was included. Treated mice weighed less (29.3 vs 55.8 g, $p < .00006$) and had smaller epididymal fat pad weight on a g (1.141 vs 3.596, $p < 0.002$) and per mg/g bw (38.88 vs 64.63, $p < 0.03$) basis vs control mice. Testis weight on a g basis was not affected by leptin, but on mg/g basis was 100% greater vs control mice (7.35 vs 3.50, $p < 0.0004$). Seminal vesicle weight was not affected. Three of four treated mice impregnated females (4/10,5/10,2/10,0/0). No control mice impregnated females. These results indicate that low doses of leptin restore fertility to *Lep^{ob}Lep^{ob}* mice.
(Support DAMD17-97-1-7055 and the Hormel Foundation)

MAILING ADDRESS OF FIRST AUTHOR
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